ROLE OF MAST CELLS IN REACTIONS OF THE BLOOD SYSTEM DURING INFLAMMATION

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Recent studies in vitro have shown that besides mediating the initial disturbances of the microcirculation and of vascular permeability, biologically active substances of mast cells (MC) in an inflammatory focus may be essential modulators of the functions of the different leukocytes [8]. However, the question of the real role of MC in leukocyte modulation in vivo and of reactions of the peripheral blood and bone marrow interconnected with it has virtually not been studied.

The aim of this investigation was to study cellular reactions of different components of the blood system under natural conditions of inflammation and after removal of the focus by MC.

EXPERIMENTAL METHOD

Experiments were carried out in the fall and winter and during the morning on 144 male CBA mice weighing 18-20 g, obtained from the "Rassvet" Nursery (Tomsk). The model of inflammation was acute infectious peritonitis caused by intraperitoneal injection of 0.5 LD_{50} *E. coli* strain ATCC 25922 in 0.3 ml of isotonic sodium chloride solution. The animals were decapitated at different stages of inflammation. The total number of leukocytes and composition of their populations in exudate and blood, the total number of myelokaryocytes per femur and the myelogram were determined. Exudate was obtained by flushing out the peritoneal cavity with 2 ml of isotonic sodium chloride solution, containing heparin 5 U/ml. To remove the MC in the peritoneal cavity 1.8-2 ml sterile distilled water was injected intraperitoneally 10 days before inflammation was induced [1, 6].

EXPERIMENTAL RESULTS

The study of the total number of leukocytes and cell composition of the exudate under natural conditions of inflammation showed biphasic accumulation of leukocytes in the focus (Fig. 1). The first, longer phase was observed during the first 5 days, with a maximum toward the 3rd day. By the 6th day the leukocyte count was below the indicated maximum, but was still higher than initially. The second, brief phase was observed on the 7th day, and by the 9th day the number of cells no longer differed significantly from the initial value. Analysis of the cell population of the exudate showed that the first phase is due to successive accumulation of neutrophils and monocytes, the second to an increased influx of monocytes. There were two "high tides" in accumulation of neutrophils. The first, which was rapid and short in duration, was observed toward 6 h, and was followed by a reduction in their number toward 12 h. The second, gradual and more prolonged, reached a peak after 2 days, and was followed by a progressive decrease in the number of neutrophils, which remained comparatively small until the end of the experiment (9 days). The number of monocytes increased significantly until 12 h, reached a peak by 3 days, and then virtually repeated the time course of the total leukocyte count, for monocytes accounted for the majority of the cell population of the exudate.

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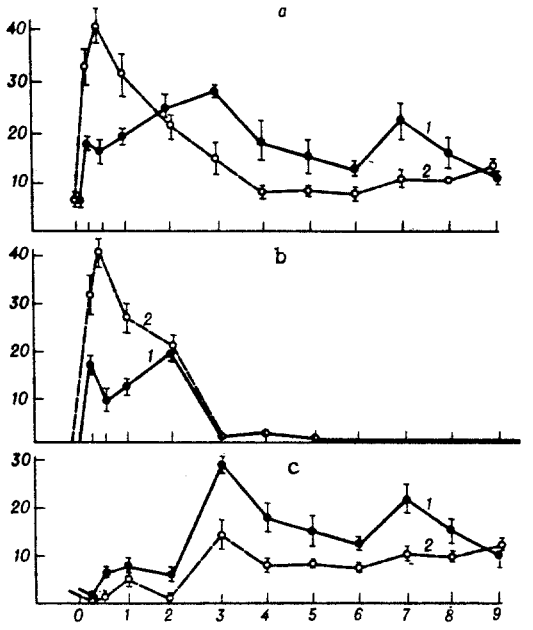

Fig. 1. Time course of total number of leukocytes (a), neutrophils (b), and monocytes (c) in peritoneal cavity of mice with acute infectious peritonitis developing under natural conditions (1) and after removal of MC from the peritoneal cavity (2). Abscissa, time after induction of inflammation (in days); ordinate, total number of leukocytes, number of neutrophils and monocytes ($\cdot 10^6$). Confidence intervals at p = 0.05.

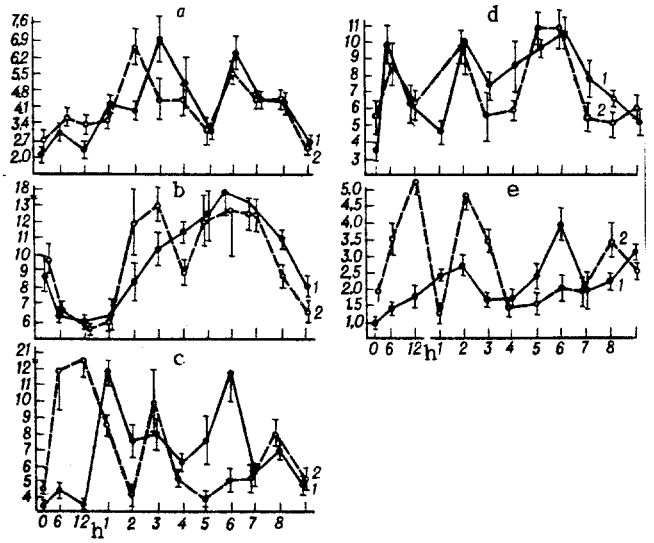

Fig. 2. Dynamics of immature (a) and mature (b) forms of neutrophils and monocytes (d) in bone marrow, of polymorphs (c) and monocytes (e) in peripheral blood of mice with acute infectious peritonitis, developing under natural conditions (1) and after removal of MC from the peritoneal cavity (2). Abscissa, time after induction of inflammation (in days), ordinate, total number of immature and mature forms of neutrophils and monocytes in bone marrow ($\cdot 10^6$), and of polymorphs and monocytes in peripheral blood ($\cdot 10^9$/liter). Confidence intervals at p = 0.05.

A marked neutrophilic and monocytic leukocytosis was observed in the peripheral blood at all times of the investigation, and this also characteristically occurred in two phases (Fig. 2). Peaks of neutrophils were observed on the 1st and 6th days, of monocytes on the 2nd and 6th days.

Marked biphasic activation of medullary granulomonocytopoiesis also was observed (Fig. 2). For instance, the maximal increase in the number of immature forms of neutrophils was recorded on the 3rd and 6th days. The time course of mature forms of polymorphs in the bone marrow differed in character. Initially there was a marked reduction in their number toward 12 h, followed by a gradual increase until 6 days. This is evidence that the first phase of leukocytosis and accumulation of leukocytes in the focus was based primarily on their arrival from the reserve pool in the medulla, and also on stimulation of granulomonocytopoiesis, as indicated by the time course of immature bone marrow cells. As regards the second phase of leukocytosis, it was clearly due to hyperplasia of the bone marrow, preceding the second wave of accumulation of leukocytes in the focus.

A study of the total number of leukocytes and their cell composition in the exudate of animals after destruction of MC likewise revealed a biphasic increase in the total number of leukocytes in the focus (Fig. 1). In this case, however, the first phase was shortened to 3 days, its peak was shifted to 12 h, and the total number of leukocytes was significantly greater than during the natural course of infection (after 6, 12, and 24 h). This was immediately followed by a rapid decrease in the leukocyte count, and starting with the 3rd day it settled down at a lower level than during the ordinary course of inflammation. By the 6th day the leukocyte count was minimal compared with the peak mentioned above. By the 7th day a tendency was found toward a fresh increase in the number of leukocytes in the focus, which was significant on the 8th and 9th days compared with the 6th day. The-first phase was due to the considerably greater accumulation of neutrophils, rising to a peak at 12 h, than under natural conditions of inflammation. Meanwhile accumulation of monocytes lagged behind that during the ordinary course of inflammation. Its peak occurred on the 3rd day. The second phase, due to an increased influx of monocytes, was late and was less marked.

Destruction of MC also led to significant changes in the time course of the blood leukocytes (Fig. 2). The earlier development of marked neutrophilia and monocytosis was observed. For instance, the first peak of neutrophilia was observed as early as after 6 h, and of monocytosis after 12 h, whereas with the natural course of inflammation they were observed after 24 h and 2 days respectively. The second peak of neutrophilia was shifted to the 3rd day, and of monocytosis to the 2nd day.

After destruction of MC earlier activation of hematopoiesis also was observed (Fig. 2). The content of immature and mature forms of neutrophilic granulocytes in the bone marrow reached peak values by the 2nd and 3rd days respectively, whereas the monocyte count on the 1st day was significantly higher than at the same time in animals with intact MC.

Thus removal of MC had a marked effect on cellular reactions of all components of the blood system during inflammation — the focus, the peripheral blood, and the bone marrow. On the whole these effects were expressed as intensification of the initial accumulation of neutrophils in the focus, the earlier and more intensive leukocytosis, and activation of granulomonocytopoiesis. Meanwhile the kinetics of monocytes in the focus was less distinct and indicated some delay of resolution of the inflammatory reaction.

Changes in kinetics of the leukocytes after destruction of MC show that under natural conditions of inflammation MC directly or indirectly exert a modulating influence on leukocytosis. It is expressed, in particular, as delay of the initial influx of neutrophils and enhancement of the influx of monocytes. Allowing for the fact that accumulation of neutrophils and monocytes is an indicator of the intensity of evolution and resolution of the inflammatory process [4], it can be concluded that the modulating effect of MC is aimed at limiting destructive processes and strengthening repair processes. Our results are evidence that if MC are removed the mortality among mice during the first 24 h after intraperitoneal injection of *E. coli* in a dose equal to LD_{50} is almost doubled. Evidently the uncontrollable massive outflow of neutrophils, the excessive release of lysosomal enzymes and, possibly also, disturbance of their other functions may lead to potentiation of septic-destructive phenomena in the focus and to the development of toxemia. Parameters of mortality and rate of resolution of the inflammatory reaction as integrative parameters allow the conclusion to be drawn that mast cells combine proinflammatory (enabling participation in the triggering mechanisms of inflammation) and control, antiinflammatory properties, and that they have on the whole a protective function.

The results are in agreement with certain data relating to the effect of biologically active substances of MC on functions of different leukocytes in vitro. It has been shown, for instance, that histamine, acting through H_2-receptors, inhibits chemotaxis, degranulation, and formation of the superoxide anion by activated neutrophils [7, 11], but increases formation of the superoxide anion and chemiluminescence of monocytes [3, 5]. Serotonin stimulates chemotaxis and chemo-

kinesis, and together with histamine, stimulates secretion of mononuclear cells. In this connection it has been suggested that products of MC in vivo may have a modulating effect both on polymorphs and on medullary hematopoiesis through the monocytes of the focus as a result of increased release of interleukin-1 [9]. Heparin of MC may have a controlling action on leukocytes. For instance, it has been shown to be able to induce aggregation of polymorphs in vitro and to inhibit leukocytic proteinases [2, 10]. The modulating effect on leukocytes may also be linked with other biologically active substances of MC, namely platelet activating factor, hydroxyeicosatetraenoic acids, and leukotrienes [12, 13], and also with nonmast-cell mediators, in the regulation of whose production MC play a role, for example, with prostaglandins [14].

The abolition of these and other control mechanisms may evidently be responsible for the effect of abolition of MC on leukocytes of the inflammatory focus described above, which in turn may affect the intensity of reactions of the bone marrow and peripheral blood through the neurohumoral mechanisms of their regulation, modulated by changes in the redistribution of leukocytes and release of their various hematopoietic factors: colony-stimulating, inducing monocytopoiesis, interleukin-1, etc. The possibility cannot be ruled out that MC may have an influence over hematopoiesis not only through modulation of the leukocytes in the focus, but also through the massive supply of biologically active substances of MC into the blood stream.

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